POLYSACCHARIDE RESOLVING GELS AND GEL SYSTEMS FOR STACKING ELECTROPHORESIS

This invention relates to electrophoretic gels. More particularly, the invention relates to electrophoretic resolving gels having improved sieving and working properties comprising one or more polysaccharides or polysaccharide derivatives, at least one of which has been partially depolymerized; electrophoretically effective discontinuous gel system combinations of resolving gels with one or more polysaccharide or derivatized polysaccharide stacking gels; and kits comprising the gel system ingredients in premeasured or hydrogel form.

Crosslinked polyacrylamide, produced by polymerizing acrylamide containing a few percent of N,N'methylenebisacrylamide, is extensively employed as the matrix for gel electrophoresis. This is due primarily to three properties of the polymer, namely: excellent mechanical strength, adherence to glass surfaces and wide control of pore size, thereby permitting fractionation of moieties ranging from simple amino acids to complex biological substances having molecular weights in the millions.

There are, however, certain attributes of cross linked polyacrylamide which detracts from its application as an electrophoretic medium. A major concern is that the gel is formed by a polymerization reaction utilizing free radicals, which is exothermic. As is well recognized, free radical reactions depend on a variety of parameters such as concentration of initiators which themselves tend toward instability, monomer purity, temperature, time, oxygen tension and absence of other inhibitors; managing these factors can require an inordinate amount of care and attention in order to achieve reproducible results. Another at least potential objection to crosslinked polyacrylamide is the possible health hazard from handling of the precursor monomers, acrylamide having been found to be a neurotoxin.

Over the past several years, a great deal of effort has been expended in the investigation and development of electrophoretic gel systems which are free of the problems associated with polyacrylamide. As a result of these research efforts, the polysaccharide agarose has emerged as a gel candidate showing considerable promise. Agarose is non-toxic, has high gel strength, low electroendosmosis and does not require free radical polymerization for gel formation. Agarose is a naturally occurring, substantially linear polymer which forms gels that are thermally reversible, thereby enabling separated components to be recovered from the melted gel.

Gels prepared with native (non-derivatized) agarose exhibit a characteristic coarse pore structure, a feature which renders them the preferred medium for the electrophoretic separation of large macromolecules. Generally speaking, primarily proteins having molecular weights in excess of about 500,000 (500 kD) can be resolved. Although smaller molecular weight entities can be resolved (restricted) by increasing the agarose content of the gel, this produces high viscosities in the agarose casting solutions, which make them very difficult to handle. Agarose gels are thus precluded from being used in a number of analytical and preparative procedures.

The large pore limitation of agarose gels can be diminished and their sieving action improved by forming

the gels from certain agarose derivatives having a finer pore structure than the parent agarose. One preferred class of such modified agarose is hydroxyalkylated agarose produced by replacing 1 to 4 hydroxyl hydrogen atoms in the agarobiose units of the agarose polymer chain with hydroxyalkyl moieties. An especially preferred member is hydroxyethylated agarose obtained by reacting agarose with 2-chloroethanol in the presence of alkali. Gels from hydroxyethylated agarose are capable of resolving proteins of from about 50 kD to about 600 kD. Moreover, such gels have lower melting points than native agarose gels, an advantage when recovering sensitive biological substances from the remelted gels.

Hydroxyethylated agarose is sold by FMC Corporation, BioProducts Group, Rockland, Me. 04841 U.S.A. under the trademark SeaPlaque®. Further details on the description and preparation of hydroxyalkylated agarose will be found in U.S. Pat. No. 3,956,273—Guiseley, which is incorporated herein by reference.

Although a decided advance in the art, the hydroxyalkylated agaroses, as with native agarose, form casting solutions whose viscosity increases with gel concentration. This makes it difficult to prepare gels of sufficient concentration to achieve maximum sieving action.

It has been reported that the sieving properties of agarose gels can be improved by combining them with other gel forming materials such as polyacrylamide; see Bode, H. J. (1977) Anal. Biochem. 83, 204–210. However, such mixtures have compatibility problems, especially when they contain high percentages of agarose. Moreover, such heterogeneous agarose blends have not afforded consistent improvement of the protein separation patterns. This is especially true when conducting submerged electrophoresis, where additives not incorporated in the gel structure have a tendency to diffuse out of the gel unless incorporated in the buffer as well. In contrast, an all agarose system forms an integrated gel.

While various agarose sieving gels are known, all sieving gels are not optimum for use as resolving gels. Examples of known sieving gels include the following.

NuSieve® GTG is an agarose sieving gel, which is a product of FMC Corporation, BioProducts Group, Rockland, Me. 04841, U.S.A., whose use has been described in "Small DNA Fragment Separation and M13 Cloning Directly in Remelted NuSieve® GTG Agarose Gels" by Dumais & Nochumson, *BioTechniques,* 5:62 (1987). Buffer systems disclosed in FMC Corporation literature as commonly used with NuSieve GTG agarose include:

TAE (40 mM Tris, 20 mM acetate, 2 mM EDTA at pH 8.0) and

TBE (89 mM Tris, 89 mM borate, 2 mM EDTA at pH 8.0).

NuSieve comprises a native agarose which has first been derivatized to hydroxyethyl agarose and has then been partially depolymerized.

Another known sieving agarose is a combination of one part SeaKem® native agarose (a product of FMC Corporation, BioProducts Group, Rockland, Me. 04841 U.S.A.) with three parts of the above described NuSieve® agarose. This combination has been disclosed as useful in Polymerized Chain Reaction (PCR) procedures by Saiki, Gelfand, Stoffel, et al., in "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase", *Science,* 239:486–491 (1988).